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Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*

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Abstract

Outdoor mesocosm experiments were used to examine the response of eelgrass communities to excess nutrient loading and reduced light that simulated coastal eutrophication. A series of replicated manipulations conducted between 1988 and 1990 demonstrated the effects of reduced available light and increased loading of nitrogen plus phosphorus on habitats dominated by eelgrass *Zostera marina* L. Shade and nutrients each significantly affected eelgrass growth, morphology, density, and biomass. We found no significant interactions between the effects of shade and the effects of nutrients on any plant characteristics except leaf length. The growth rate of individual eelgrass shoots was linearly related to light, increasing throughout the range of available light. Biomass and daily biomass increase, or areal growth, were also linearly related to light, but specific growth showed no response to light. Shoot density increased with the log of light.

Excess nutrient loading was shown to significantly reduce eelgrass growth and bed structure through stimulation of various forms of algae that effectively competed with eelgrass for light. The absence of significant interactions between the effects of shade and nutrients on eelgrass density, growth, and biomass suggests that the negative effect of algae on eelgrass occurs primarily through the reduction of light (i.e. shading). The outcome of nutrient enrichment was a shift in plant dominance from eelgrass to three algal forms: phytoplankton, epiphytic algae, and macroalgae. We quantified the effects of eutrophication and demonstrated that increased nutrient loading results in less light for eelgrass and that eelgrass growth linearly decreases with reduced light.

Eutrophication, caused by increased nutrient loading, is widely acknowledged to impact estuarine communities dominated by eelgrass, *Zostera marina* L. (Kemp et al. 1983; Orth and Moore 1983), primarily through changes in plant species composition (Borum 1985; Twilley et al. 1985; *see also* van Montfrans et al. 1984). These field studies, from both Europe and the U.S., describe losses of eelgrass resulting from eutrophication and often implicate shading from various algal forms.

Investigations into the effects of shading on both temperate and tropical seagrasses have focused primarily on the kinetic response of individual plants or leaf pieces to reduced light conditions (*see* Sand-Jensen 1977; Drew 1979; Fourqurean and Zieman 1991) or on field manipulation of light levels (Backman and Barilotti 1976). However, these studies either do not look at the whole-plant response to light reduction or, in examining whole plants, do not quantify morphological or growth responses to reduced light. The effects of shading by epiphytes (Sand-Jensen 1977; Orth and van Montfrans 1984) and phytoplankton (Borum 1985; Sand-Jensen and Borum 1991) have also been investigated, but the response of eelgrass bed structure and growth to algal shading was not quantified.

The direct effects of increased nutrients on eelgrass have been observed in the field through experiments that increased sediment nutrients and showed greater leaf length at higher nutrient levels (Orth 1977). Water column nutrient enrichment, similar to the nutrient loading associated with eutrophication, adversely affected eelgrass through stimulated algal growth (Harlin and Thorne-Miller 1981). Harlin and Thorne-Miller (1981) demonstrated algal growth in response to nutrient addition to the water column, but again did not quantify eelgrass morphology and growth in response to nutrient loading. Several investigators have used micro- and mesocosms to examine the effects of nutrient enrichment on eelgrass and tropical seagrasses. For eelgrass, Burkholder et al. (1992) reported

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direct nitrate toxicity. Neckles et al. (1993) found that eelgrass production in summer was reduced by nutrient enrichment that caused heavy epiphyte loads in the absence of epiphyte grazers. Similarly, Williams and Ruckelshaus (1993) found that eelgrass growth is affected by sediment nutrient resources, epiphyte load, and epiphyte grazers. Tomasko and Lapointe (1991) demonstrated increased epiphyte loads with nutrient enrichment and an interactive effect of shade and nutrient addition on *Thalassia testudinum* (decreased nutrient effect with increased shade). No studies have quantitatively examined the mechanisms by which nutrient loading affects eelgrass and eelgrass community structure.

We used mesocosms to experimentally examine the mechanisms responsible for eutrophication-related eelgrass decline. We separately measured the effects of shade and nutrients, as well as their interaction, and quantitatively assessed their impact on eelgrass growth, morphology, and biomass and on community structure. Our mesocosm experiments demonstrate that the primary impact of nutrient addition is the indirect effect of shading by algae and quantitatively relate reduced light to eelgrass decline.

Methods

The mesocosm apparatus we used consisted of 1.5 m^2 (0.8 m^3) tanks supplied with running seawater and freshwater. These outdoor tank cultures were established with natural light regimes and ambient temperature conditions. Seawater was pumped from Little Bay at a depth of 12 m into a seawater system and gravity fed to each mesocosm tank at 30 liters h^{-1}. Freshwater was supplied from a local well to the tanks at 30 liters h^{-1}. Eelgrass can become infected and be killed by the pathogenic slime mold *Labyrinthula zosterae,* which causes the wasting disease (Muehlstein et al. 1991). Since higher salinities promote infection and rapid eelgrass decline from the wasting disease (Burdick et al. 1993), salinity was held at 12–15‰ in the mesocosms.

Nutrient loading rates in the unenriched, or ambient, treatment were calculated as N and P inputs from the average weekly ammonium and phosphate concentrations entering the tanks in the inflowing freshwater and seawater. Loading rates for the enriched treatments were achieved by allowing known amounts of nutrients to diffuse into the water column from suspended mesh bags containing slow-release Osmocote fertilizer (15% ammonium and 8% phosphate by weight). Calculation of loading for the enriched treatments included measurements of nutrient levels of the inflowing water and weight loss of the fertilizer additions. The N:P ratio of the loading rates for the unenriched (control) treatments was 1.37:1. The physical structure of each mesocosm included current velocities of 2–20 cm s^{-1}, water depth of 30 cm, a flushing rate of 2 volumes per day, and sediment (15-cm depth) with a mud to sand ratio of 1:1.

Eelgrass growth rates are largely a function of light and nutrients. Because coastal pollution has a major impact on light and nutrient availability to eelgrass, these factors were manipulated in the mesocosms to assess the impact of pollution-induced stresses on eelgrass populations. In a shade experiment, irradiance was reduced from natural levels (94% of surface light at 1-cm depth) with neutral density screens. For a nutrient enrichment experiment (nutrient experiment), levels of N and P available to the plants were altered by addition of N plus P to the water column. And in a multiple factor experiment (shade by nutrient experiment), two nutrient levels were combined with three shade levels in a factorial treatment arrangement.

Shade experiment: Effects of reduced light—A mesocosm experiment was designed to examine the effects of reduced light intensity on the density, biomass, growth, and morphology of eelgrass, *Z. marina.* In 1988, six outdoor tanks were planted with eelgrass shoots in early June at a density of 200 shoots per tank. Mud snails (*Ilyanassa obsoleta*) were added in densities of 400 snails per tank. Several sticklebacks (*Apeltes quadracus* and *Gasterosteus aculeatus*) were added to control naturally recruiting amphipods. In mesocosms with high epiphyte production, amphipod numbers can increase rapidly, deplete epiphyte standing stock, and thence direct their appetites to eelgrass leaves, completely destroying the eelgrass population. Amphipod damage to eelgrass occurs in nature but appears to be limited to small portions of the leaf edges. One of our experimental tanks (50% light) was impacted by amphipod grazing and the results are not included in our analysis.

Light levels below natural intensity were achieved by covering the tanks above the water level with neutral density screen 1 week after planting. Light levels of 11, 21, 41, 61, and 94% surface light were quantified with a LiCor 4π quantum sensor. Plants were allowed to grow to maturity under the five light levels. It should be noted that the shading of these plants had no effect on the photoperiod; only the effects of reduced light intensity reaching the eelgrass leaves were examined. Reduction in light intensity by shading is analogous to decreased water clarity but not necessarily to changes in depth, since with increases in depth, changes in water color and photoperiod (unchanged in our experiment) can reduce the quality as well as the quantity of light reaching the plants (Short 1980). Changes in eelgrass morphology and physiology observed in the field over a depth gradient (Dennison and Alberte 1985) incorporate these multiple factors.

During the 4-month shade experiment, leaf growth and bed structure (including shoot density, number of leaves per shoot, leaf length, and leaf width) were measured every other month by counting shoots within a 0.04-m^2 quadrat in comparable high and low current areas of each tank and by harvesting five shoots per tank (Short 1987). Biomass of live leaves was calculated by multiplying eelgrass density by the average shoot weight taken from the growth measurements.

Nutrient experiment: Effects of nutrient loading—In 1989, a mesocosm experiment was performed to quantitatively evaluate the effects of increased nutrient loading

Table 1. Mean monthly nutrient concentrations and standard errors (SE) for ambient and enriched treatments in mesocosms during the 1990 light-by-nutrient factorial experiment.

	Ammonium (μM)		Phosphate (μM)	
	Ambient (SE)	Enriched (SE)	Ambient (SE)	Enriched (SE)
Aug	8.26(1.58)	10.67(2.41)	0.70(0.09)	1.38(0.21)
Sep	4.46(0.44)	7.45(1.85)	0.50(0.09)	1.42(0.31)
Oct	3.25(0.64)	10.98(2.33)	0.68(0.11)	2.35(0.22)
Nov	3.21(0.62)	13.96(5.41)	0.74(0.13)	2.22(0.50)

on eelgrass beds. Six mesocosms were planted with 200 eelgrass shoots each and grown under ambient conditions for 2 months. Animals were added to each tank in order to include the major organisms that would create a balanced ecosystem. Mud snails (*I. obsoleta*) were added in densities of 400 snails per tank. Carnivorous fish were added to control amphipods: sticklebacks (*A. quadracus* and *G. aculeatus*) 20 per tank and pipefish (*Syngnathus fuscus*) 4 per tank. After 2 months, the tanks were paired according to eelgrass density, with one of each pair a control and the other enriched with N and P. Beginning 8 August, nutrients were added continuously in the form of slow-release Osmocote fertilizer at a level producing concentrations elevated above ambient nutrient concentrations as determined by initial ammonium (Koroleff 1976) and phosphate (Strickland and Parsons 1972) analyses of the water column. Nitrogen loading rates (260 mg N d^{-1}) were calculated at 6.5 times the N received by the ambient treatment tanks (40 mg N d^{-1}) with N:P ambient ratio of 1.3:1 and an enrichment ratio of 1.2:1.

Leaf growth and bed structure were measured every other month (as above) by harvesting 10 shoots per tank, but shoot densities were determined monthly from 4 quadrats of 0.02 m^2 in comparable high and low current areas (two each) of each tank. After 4 months of continuous nutrient additions, the experiment was terminated and final biomass, shoot density, and other plant characteristics were measured.

Shade by nutrient experiment: Interactive effects of reduced light and nutrient loading—In 1990, an experiment was designed to simultaneously quantify the effects of both reduced light and increased nutrients on eelgrass beds, thereby testing for shade by nutrient interaction. Twelve eelgrass community mesocosms were set up as in the nutrient loading experiment and subjected to three shade (11, 41, and 94% surface) and two nutrient loading levels (ambient and 6× ambient) in a factorial treatment arrangement of two replicates for each treatment combination in a completely randomized design. The uptake of nutrients from the water column by eelgrass and algal forms reduced nutrient concentrations so that they ranged from 2 to 5 times ambient in the enriched tanks (Table 1).

Weekly shoot densities were estimated from three 0.02-m^2 quadrats in comparable high, average, and low current areas of each tank and averaged for each month (August through November). Monthly means of shoot densities were analyzed by repeated measures ANOVA. Nutrients in the inflowing water were monitored weekly, as before. Eelgrass morphology and growth were estimated for each tank in October and November by marking and harvesting 10 shoots (Short 1987), and the data were analyzed by blocking on month. Aboveground biomass estimates for October and November were generated from shoot density measurements and shoot weights obtained from growth measurements. A final biomass measurement was made at the close of the experiment in late November when all macroscopic plant material was sorted, dried, and weighed. All three estimates of biomass were combined in a repeated measures ANOVA of shade and nutrient effects.

Macroalgal biomass was assessed from a 0.0625-m^2 quadrat in comparable high and low current areas of each tank. All visible algae were removed, dried, and weighed. For measurement of epiphyte biomass, 10 eelgrass shoots were removed from the sediments, held upside down (individually) over a small bucket, hand-wiped down the length of each blade, and rinsed. The epiphyte-water slurry collected was then passed through preweighed filter paper, the filters were dried and weighed, and epiphyte biomass was calculated in terms of substratum area from concurrent shoot density measurements. Phytoplankton, reported as mg Chl *a* $liter^{-1}$, was determined from 500 ml of tank water with standard methods (Strickland and Parsons 1972). Snails and fish were initially added to each tank as in the previous year, and fish populations were increased as necessary to regulate amphipod populations.

Statistical least-squares analyses were conducted separately on the results of each experiment. Values and means of subsamples were analyzed by regression in 1988, when replicate tanks were not used. The means of replicate tanks were analyzed within an ANOVA framework in 1989 (three replicates, blocked on shoot density) and 1990 (two replicates per treatment combination). Dependent variables were log-transformed to produce homogeneous variance when indicated by residual analysis (Netter et al. 1985). Levels of type 1 error (a conclusion that there is an effect when none actually exists) are set at 0.05 for main effects and 0.10 for interactive effects so that we may be more conservative with respect to type 2 error (a conclusion that there is no effect when one actually exists). Details of specific analyses are reported in the figure legends.

Results

Shade experiment—A marked difference in shoot density among shade treatments became apparent as the 1988 season progressed (shade by date interaction: $P < 0.10$). Shoot density in July and November showed logarithmic increases with increased light to a maximum density of >400 shoots m^{-2} in November (Fig. 1a). Eelgrass density declined only at the lowest light level (Fig. 1a).

Differences in leaf size developed among the treat-

ments, with the plants at the lowest light levels (heavy shade) producing longer leaves than the plants at the higher light levels ($P < 0.05$; Fig. 1b). In all cases, leaf length in the mesocosms exceeded water depth, and some plants grew with the distal portion of the leaves horizontal to the water surface. Standing leaf biomass, which combines plant size and density, was greater with increased light ($P < 0.01$; Fig. 1c). The increased shoot density at high light conditions overwhelmed the effect of longer leaves at lower light levels, resulting in greater standing leaf biomass with increased light.

Leaf growth, measured as leaf elongation on a per shoot basis, showed a significant linear increase with increased light intensity ($P < 0.01$; Fig. 2a). However, specific growth rate (mg of new leaf per mg of shoot per day) varied little under the different shade treatments ($P > 0.10$; Fig. 2c). Leaf growth per shoot was converted to growth on an areal basis (g m^{-2}) using shoot density. By combining the effects of increased density and increased growth per shoot, we demonstrate a strong positive relationship between areal leaf production and light ($P < 0.01$; Fig. 2b).

Nutrient experiment—In the 1989 enrichment experiment, the effect of excessive nutrient loading on eelgrass populations was most evident in the reduction of shoot density and biomass observed in the enriched tanks as compared to control tanks (Fig. 3a,c). Nutrient enrichment decreased eelgrass shoot densities by 50% ($P < 0.01$), and the effect increased over the course of the experiment ($P < 0.10$). By the end of the experiment, the average eelgrass shoot biomass in the enriched tanks was reduced to a third that of the controls ($P < 0.05$). Leaf length decreased by more than 20 cm in enriched eelgrass tanks ($P < 0.05$; Fig. 3b). Note that the effects of enrichment were similar to those of light reduction for shoot density and biomass but opposite for leaf length (Figs. 1 and 3). Interestingly, the enrichment effects on eelgrass shoot density, biomass, and leaf length were similar for all replicates, even though each replicate was dominated by one of the three different algal forms: epiphytes, macroalgae, and phytoplankton.

Shoot density increased greatly between August and September ($P < 0.0001$) and then declined through the end of the experiment ($P < 0.05$). Note in Fig. 3a that the tanks were paired just before treatments began, following the August density measurements. Pairing the tanks for the statistical analysis did not reduce the variance in density or biomass when nutrient effects were tested, perhaps because the pretreatment densities as well as the block differences were small compared to later densities (Fig. 3a). On the other hand, pairing the tanks was important for analysis of leaf length and growth, which were sampled soon after pairing.

The response to nutrient addition included a reduction in eelgrass areal growth rate ($P < 0.05$; Fig. 4b) after both 1 and 3 months of enrichment. No differences between treatments were seen in growth measured on a per shoot basis (Fig. 4a). Although eelgrass responded to nutrient addition by becoming shorter and less dense (Fig. 3), the specific growth rate of the enriched treatments was slightly *greater* than that of the unenriched treatments in November ($P < 0.05$; Fig. 4c).

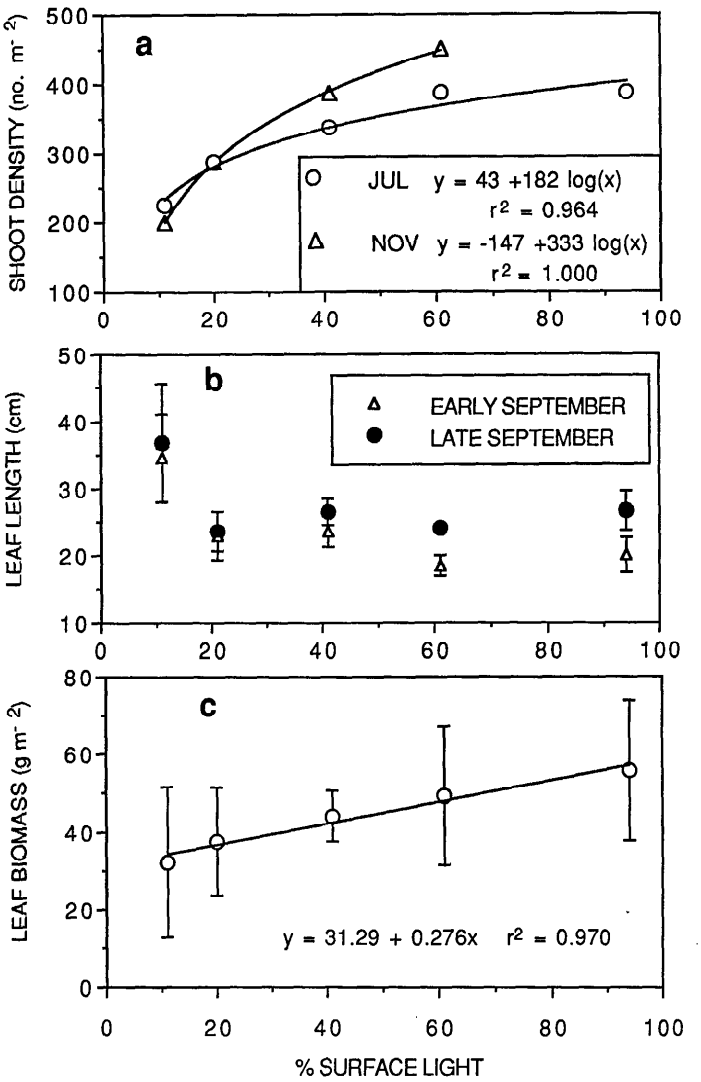

Fig. 1. Eelgrass population characteristics developed under control and four reduced light levels in mesocosms, 1988. Data are plotted as percentage of surface light at 1-cm depth vs. shoot density, leaf length, and leaf biomass. Simple linear regressions were performed on the log of light for shoot density and on untransformed light for leaf biomass. The regression coefficients were significant for density ($P < 0.01$) and biomass ($P < 0.01$). July shoot densities were an average of two 0.04-m^2 quadrats; November shoot densities were determined for the entire tank. Leaf biomass was calculated for July from density and shoot weight data. Five shoots were used to generate a mean leaf length (± SE).

Shade by nutrient experiment—The 1990 fully replicated shade and nutrient factorial experiment examined both single factors as well as interactions. Shoot densities were similar with respect to the various shade and nutrient treatments in August, 3 weeks after beginning the treatments (Fig. 5), but the overall mean of 200 shoots

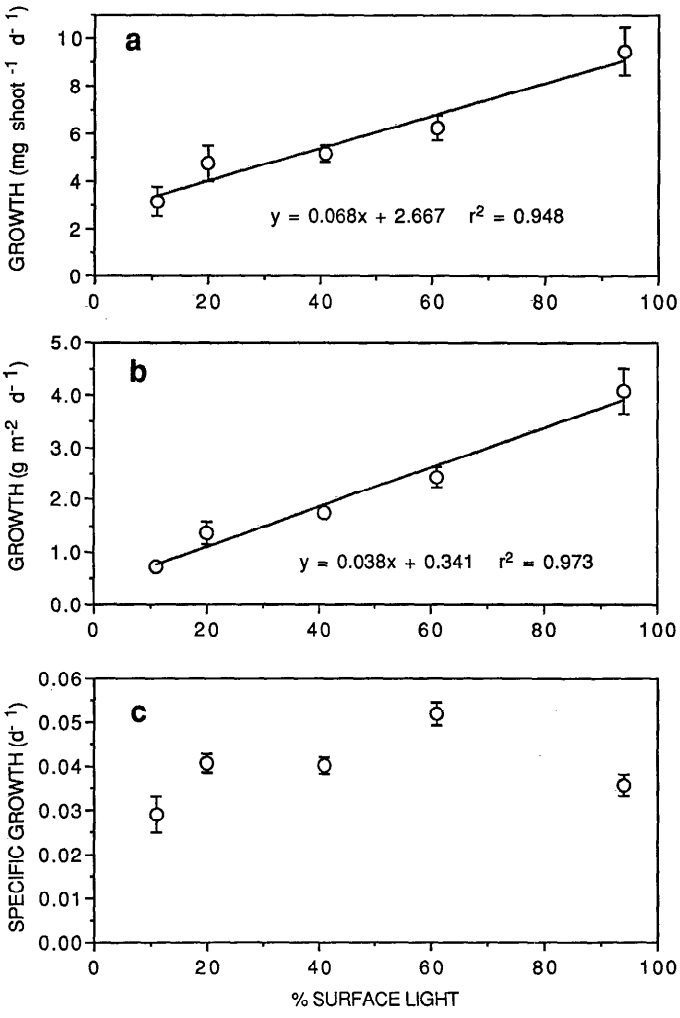

Fig. 2. Eelgrass growth rates showing means (±SE) of five shoots under control and four reduced light levels (percentage of surface light at 1-cm depth) in mesocosms, 1988. [a.] Simple linear regression performed on the mean growth of five shoots and light level showed a significant relationship ($P < 0.01$). [b.] Simple linear regression performed on the mean areal growth and light level showed a significant relationship ($P < 0.01$). [c.] Specific growth had no significant relationship with light ($P > 0.10$).

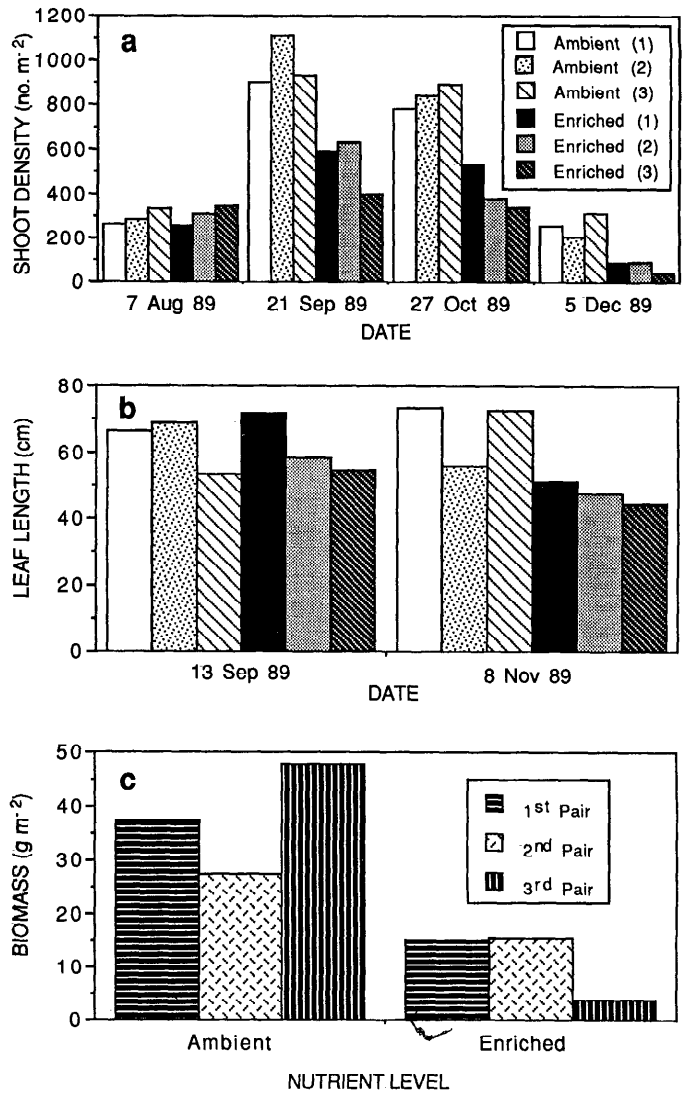

Fig. 3. Characteristics of eelgrass populations grown in mesocosms under ambient and enriched (six times ambient) nutrient treatments for each of the three replicates are shown. Tanks 1–3 of each treatment were paired according to density in early August 1989. After ANOVA with blocking on initial densities, shoot densities, determined from means of four quadrats of 0.02 m^2, were significantly greater under ambient nutrient levels ($P < 0.01$); leaf lengths, determined on 10 terminal shoots for each tank, were significantly greater under ambient nutrient levels ($P < 0.05$); and final biomass (live shoot biomass at the close of the experiment in early December) on a dry weight basis was significantly greater under ambient nutrient levels ($P < 0.05$).

m^{-2} was greater than the initial planting density of 133 m^{-2}. Shoot densities continued to increase until October (one high-light ambient-nutrient replicate reached a maximum of >600 m^{-2}) and then declined by the close of the experiment in late November (Fig. 5). Significant month-by-shade ($P < 0.05$) and month-by-nutrient ($P < 0.01$) interactions indicate that over time, decreased light and elevated nutrients both served to reduce eelgrass densities. No significant interactions between shade and nutrients were found, indicating there was no synergism between the detrimental shade and enrichment effects on eelgrass density.

Differences in plant morphology are shown by the average leaf length (excluding the youngest leaf) obtained during the growth measurements. The effect of nutrients on leaf size was significant ($P < 0.05$); plants were smaller in the enriched treatment (Fig. 6a). Also, the interaction effect of nutrient by shade was significant ($P < 0.10$). Leaves tended to be longer with decreasing light under unenriched conditions, but leaf length in enriched treatments showed no trend with reduced light (Fig. 6a).

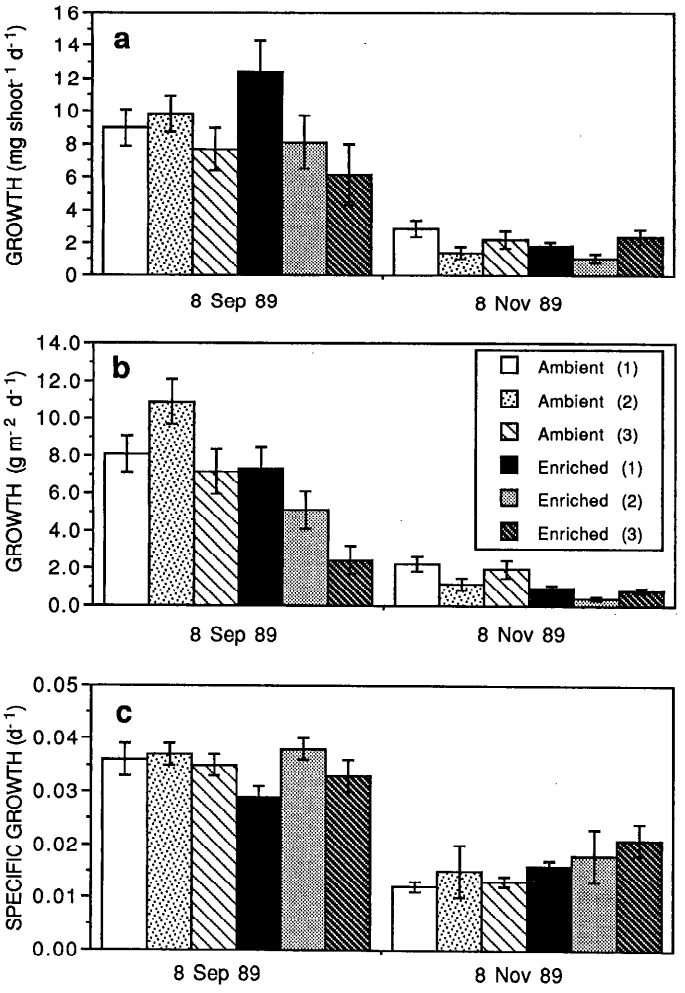

Fig. 4. Eelgrass growth rates on (a) a per shoot basis, (b) an areal basis, and (c) as specific growth under ambient and enriched conditions. Values from each of the three replicates are means from 10 terminal shoots (± SE). Differences between treatments were significant at the 0.05 level for areal growth and for specific growth in November.

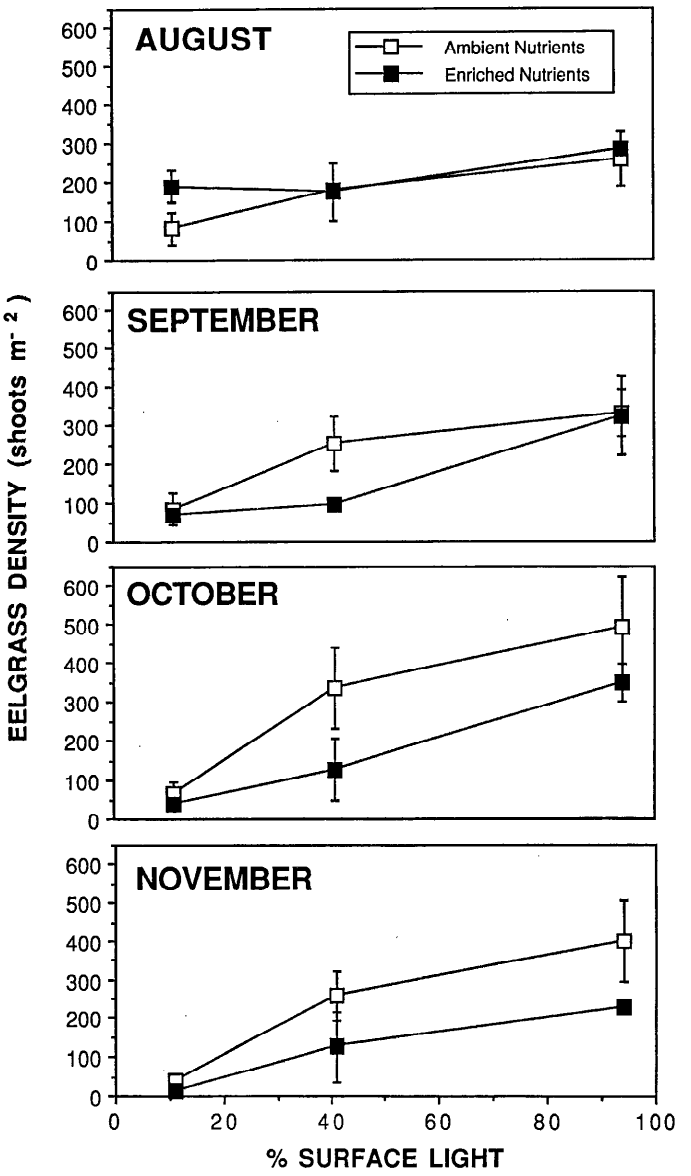

Fig. 5. Monthly eelgrass shoot density in mesocosm tanks during the shade-by-nutrient experiment, 1990. Values are the mean of two replicate tanks where shoot densities in three 0.02-m^2 quadrats per tank taken on each of three to five sampling dates per month (± SE). Using ANOVA, we found date-by-light ($P < 0.05$) and date-by-nutrient ($P < 0.01$) interactions to be significant, indicating that both light and nutrient effects became significant over time.

Aboveground biomass estimates for October and November and the destructive harvest at the close of the experiment in late November were combined in a repeated-measures ANOVA using log-transformed data to correct for nonhomogeneity of variance. Shade ($P < 0.001$) and nutrient ($P < 0.05$) effects were significant, but their interaction was not ($P > 0.10$), indicating that there was no synergism between these two factors controlling shoot biomass (Fig. 6b). Nutrient loading at six times ambient levels reduced biomass by >50% at all light levels (Fig. 6b). Decreasing light from 94 to 11% of surface levels reduced shoot biomass 27-fold at both nutrient levels. For all the treatment combinations, biomass declined almost 50% from October to the final measurement in November ($P < 0.01$); this seasonal effect was stronger at lower light levels ($P < 0.05$ for the interaction between shade and month effects).

The outcome of nutrient loading on the various plant components of the eelgrass community was assessed by comparing the biomass of the three algal competitors to that of eelgrass. In full sunlight (94%) under ambient loading, eelgrass biomass dominated the epiphytic, macroalgal, and planktonic components (Table 2). However, under enriched conditions both the full light replicates became dominated by a mix of algal forms. The largest biomass of plant tissue was in the form of macroalgae

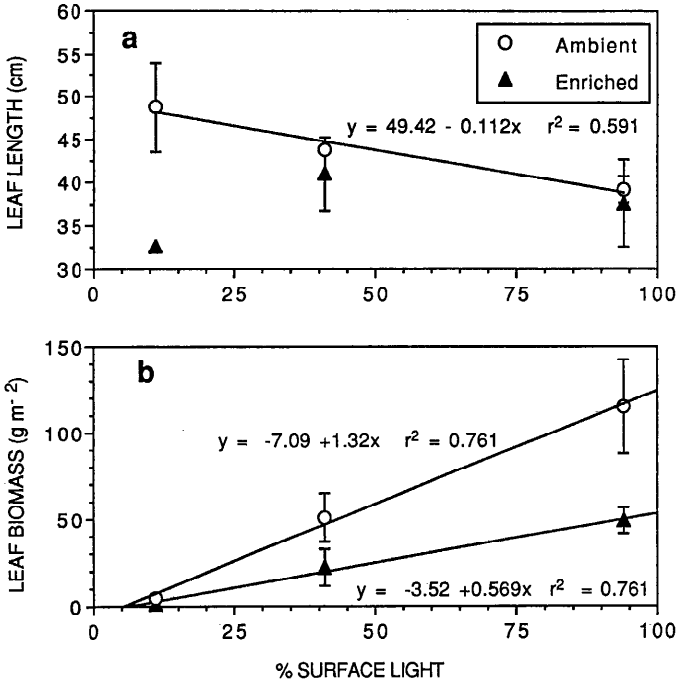

Fig. 6. Leaf length and biomass of eelgrass in the shade-by-nutrient experiment. [a.] Leaf length values are the means (±SE) of two replicate tanks (10 shoots per tank harvested in October 1990). ANOVA indicated that nutrient loading ($P < 0.05$) and the nutrient-by-light interaction ($P < 0.10$) were significant. Leaf length increased with reduced light ($P < 0.10$) only in the unenriched tanks. [b.] Average eelgrass leaf biomass on a dry weight basis from two estimates (based on average shoot weight and shoot density in October and November) and direct biomass determination at the close of the experiment in November. Values are means (± SE) of two replicate tanks over the three dates. Using repeated-measures ANOVA we found light ($P < 0.01$) and nutrient loading ($P < 0.05$) to be the only significant effects. Biomass was log-transformed to reduce error variance over its range.

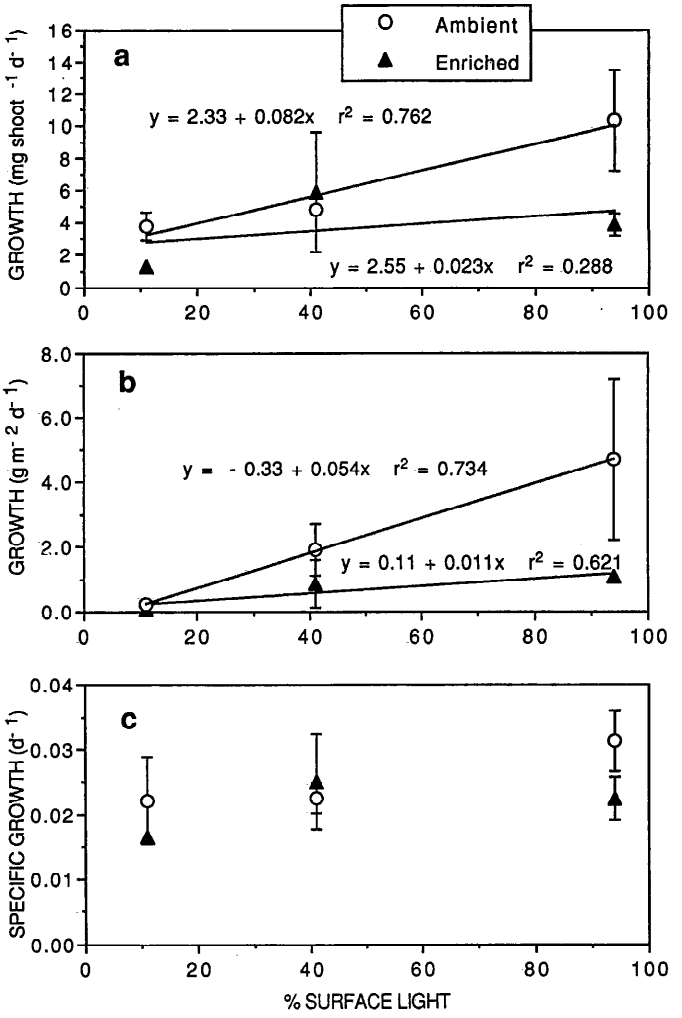

Fig. 7. Eelgrass growth rates measured in October on (a) a per shoot, (b) an areal, and (c) specific growth basis for the shade-by-nutrient experiment, 1990. Values are means (± SE) of two replicate tanks. ANOVA with blocking on month indicated that both light and nutrients were significant for areal growth ($P < 0.01$) and for per shoot growth ($P < 0.05$), but neither was significant for specific growth ($P > 0.10$). Growth per shoot and areal growth were log-transformed to reduce error variance over their ranges. To improve interpretability, we back-transformed equations, but r^2 represents the fit of the data to the model under transformation.

(Table 2), although heavy epiphytic algal growth on the eelgrass leaves and substantial phytoplankton populations were also present.

Because eelgrass growth for October and November was measured on plants that were destructively harvested, data were analyzed by blocking on month. On a per shoot basis, growth decreased substantially with shading and nutrient loading (Fig. 7a) and was almost 7-fold less in November than in October. The effects of month ($P < 0.0001$), shade ($P < 0.05$), and nutrient loading ($P < 0.05$) were significant, but there were no significant interactions. Thus, the trends in growth due to shade and nutrient treatments did not change from October to November, and only October data are shown.

When examined on an areal basis, eelgrass growth was significantly influenced by month ($P < 0.001$), shade ($P < 0.0001$), and nutrients ($P < 0.01$), but again no interactions were found. Growth was over six times greater in October than in November and decreased substantially with shading and nutrient loading (Fig. 7b). These are the same trends found for the analysis of growth on a per shoot basis. Note that growth on an areal basis would all but cease at 11% light, as shoot density approaches zero (Figs. 5 and 7b).

Specific growth, the amount of new leaf tissue compared to the total shoot biomass, did not differ significantly with shade or nutrient treatments, but declined almost 5-fold ($P < 0.0001$) from October (Fig. 7c) to November (mean specific growth = 0.0047 d^{-1}). Because this measure removes the effects of shoot density and plant size from growth, it implies, as in the earlier experiments, that the plants have adapted to grow at similar

Table 2. Community response of plants in eelgrass mesocosms to nutrient enrichment at three light levels (94, 41, and 11%) in the shade-by-nutrient experiment, 1990. Biomass values are means (SE) of two replicate mesocosms on a dry weight basis. Eelgrass shoot biomass is based on the mean weight of 10 terminal shoots and tank densities in October, when competitors were sampled (500 ml for phytoplankton, 10 shoots for epiphytes, and a 0.0625-m^2 quadrat for unattached macroalgae). Percentages of control conditions are calculated from the biomass data by dividing by the data for full light (94%) and ambient nutrient conditions.

	Ambient nutrients			Enriched nutrients		
	94%	41%	11%	94%	41%	11%
Plant biomass						
Eelgrass shoots, g m^{-2}	117(54)	83(36)	15(12)	41(4)	20(10)	5(1)
Phytoplankton, mg Chl *a* $liter^{-1}$	2.2(0.2)	2.3(0.9)	0.9(0.2)	3.0(0.6)	2.4(0.4)	0.9(0.1)
Epiphytes, g m^{-2}	1.6(1.6)	13.3(12.7)	0.1(0.1)	20.3(0.0)	4.4(4.0)	0.5(0)
Macroalgae, g m^{-2}	0(0)	0(0)	0(0)	179(8)	0(0)	0(0)
Percent of controls						
Eelgrass shoots	100	71	13	35	17	4
Phytoplankton	100	105	40	136	109	40
Epiphytes	100	809	8	1,267	266	29
Macroalgae	100	0	0	17,900*	0	0

* Control assigned a value of 0.01 g.

specific growth rates regardless of light intensity but may have slightly elevated specific growth rates with nutrient additions.

Discussion

Our mesocosm studies quantitatively demonstrate the effects of reduced light and increased nutrient loading on eelgrass at several levels: eelgrass morphology, growth response, and plant community dynamics.

Eelgrass morphology and canopy structure—The increase in leaf length in the 1988 shade experiment appears to be a morphological adaptation of the plants to reduced light intensity. The eelgrass leaves grew to greater lengths under low light conditions, but shoot density decreased (Fig. 1). The combination of leaf length and shoot density changes in response to light resulted in a positive relationship between leaf biomass and light. The finding of greater leaf length with reduced light in 1988 was supported in the 1990 shade-by-nutrient experiment, in which the shade effect on leaf length was evident in the ambient nutrient treatment (Fig. 6a). Additionally, the relationship of decreased shoot density to low light was apparent by September 1990 (Fig. 5). Thus the eelgrass bed structure, defined by density and shoot length, is likely controlled by the availability of light in noneutrophic waters. Differences in leaf length and density have been observed along a depth transect in the field that confounded differences in the quality as well as the quantity of light (Dennison and Alberte 1985). It is clear that decreasing light intensity only, which is analogous to decreasing water clarity, has a major effect on eelgrass production, standing biomass, and bed structure. Despite the shallow water and absence of tides in the mesocosms, the plants responded to decreased light levels with lower shoot density and biomass production but greater leaf length.

The response of eelgrass leaf length to excess nutrient loading was opposite to its response to light, resulting in shorter leaves with excess nutrient loading (Figs. 3b and 6a). Previous research has shown greater plant size associated with somewhat elevated nutrient resources (Orth 1977; Short 1983, 1987), but these studies dealt with sediment nutrient supply and did not test the effects of excess nutrient loading to the water column, which stimulates the growth of algal competitors. In the enriched mesocosms, algal fouling of the leaves might result in either early leaf loss (though there was no statistically significant effect of nutrient enrichment on leaf number) or a greater leaf turnover rate, so that the older, longer leaves are lost, resulting in shorter plants. Experiments in which nutrient addition alone stimulates a shade-creating condition above the eelgrass canopy produce an eelgrass response in which leaf length changes are complicated by the interaction of the opposing effects of shade and nutrient loading. In these cases (nutrient and shade-by-nutrient experiments), excess nutrients resulted in shorter leaves, suggesting that negative impacts on plant morphology, quite different from those of shading, accrue from excess nutrients.

The effects of shade and nutrients together (shade-by-nutrient experiment) on density, biomass, and growth of eelgrass were similar to the results of the shade and the nutrient experiments, and no shade-by-nutrient interactions were found except with leaf length (as above). Our mesocosm experiments clearly demonstrate that the primary negative effect of nutrient loading on eelgrass is not a direct effect but is rather the stimulation of algal competitors that have the indirect effect of reducing the light available for eelgrass growth and survival.

The relationship between eelgrass density and light showed a shift to reduced densities at low light in both the shade and shade-by-nutrient experiments (Figs. 1 and 5). Because eelgrass densities were still decreasing in November at 11% light (but were steady at 21% light), it is questionable whether eelgrass beds could sustain them-

selves throughout the year at 11% of surface light. Overall, our experimental findings support previous work suggesting that the minimum light limit of eelgrass survival is between 10 and 20% surface light (Duarte 1991).

Growth response—The response of seagrass growth to reduced light conditions has typically been described by a hyperbolic function demonstrating saturation kinetics (e.g. Drew 1979; Fourqurean and Zieman 1991). However, these measurements were made in short-term experiments under laboratory conditions and not with intact plants. The light-saturation conditions found in previous studies did not use plants grown under the test light treatments, and thus the plants were not morphologically adapted to these conditions. Our growth measurements were made on adapted plants that had adjusted their physiology and morphology to the specific shade condition being tested, much as plants adapt in nature. The results of both the 1988 shade experiment and the 1990 shade-by-nutrient experiment show a significant linear response of eelgrass growth to light on both a per shoot and an areal basis (Figs. 2 and 7). This linear response is in contrast to hyperbolic functions that show eelgrass growth reaching light saturation. The continuous linear growth increase found here suggests that eelgrass plants use all available light up to full sunlight to increase their productivity once they have adapted their morphology to their environment. The linear growth response to reduced light conditions is similar to the linear response of leaf biomass (Figs. 1c and 6b).

Under elevated nutrient concentrations in the 1989 nutrient experiment, the growth of eelgrass was significantly lower on an areal basis (Fig. 4b). Similarly, in the 1990 shade-by-nutrient experiment, the growth of eelgrass on both a per shoot and an areal basis showed a reduction at elevated nutrient concentrations (Fig. 7a,b). The absence of a shade-by-nutrient interaction indicates that the primary effect of nutrient enrichment was not physiological but the indirect result of shading. Although eelgrass is often considered to be nutrient limited in its growth (Short 1987), our experiments show that water column nutrients in excess of eelgrass requirements stimulate the growth of competitive algae (Table 2), diminishing eelgrass growth and biomass (Figs. 3c and 6b).

An unexpected result of this study was the evidence that eelgrass adapts to maximize specific growth rate at all light levels above ~20% surface light by adjusting plant morphology and shoot density. For a given time of year, eelgrass showed no differences in specific growth rates due to shading. Nutrient enrichment, on the other hand, may stimulate specific growth rates. Such stimulated specific growth rates, coupled with reduced areal production, biomass, and plant size may be useful as a stress indicator of excessive nutrient loading to an eelgrass population. Thus, based on our studies, any management action to increase water clarity or decrease nutrient loading from surrounding uplands will increase eelgrass productivity and improve the health of an estuary, specifically by improving eelgrass bed structure, filtration capacity, and secondary production (Dennison et al. 1993; Short et al. 1993).

Plant community dynamics—The stimulation of planktonic, epiphytic, and macroalgal growth in response to excess nutrients (bottom-up control) has been studied in many marine environments (Cambridge et al. 1986; *see* Elmgren 1989). Additionally, the ability of opportunistic algal forms to replace existing seagrass communities during nutrient loading conditions has been documented (Harlin and Thorne-Miller 1981; Borum 1985; Tomasko and Lapointe 1991). In our 1989 nutrient experiment, identical enrichment conditions resulted in one mesocosm tank becoming phytoplankton dominated, one epiphyte dominated, and one macroalgae dominated. In each case, eelgrass habitat structure (measured as a combination of shoot density and leaf length; Fig. 3) declined. The production of these various algal forms competing with eelgrass for light under enriched conditions in our 1990 shade-by-nutrient experiment (Table 2) again resulted in decreased eelgrass habitat structure (Figs. 5 and 6). Similar results have been found in other eelgrass experiments (Burkholder et al. 1992; Neckles et al. 1993; Williams and Ruckelshaus 1993). Such a condition in nature likely leads to a reduction in material and energy flows from primary to secondary producers and to higher trophic levels (Elmgren 1989; Kautsky 1991).

Excessive nutrient loading in an estuary eliminates the eelgrass community by pushing the eelgrass system toward dominance by one of the three algal competitors, as observed along the east coast of the U.S., including Chesapeake Bay (Kemp et al. 1983), Ninigret Pond, Rhode Island (Harlin and Thorne-Miller 1981), and Waquoit Bay, Massachusetts (Costa et al. 1992; Short et al. 1993). Although direct nutrient uptake by eelgrass leaves at high nutrient concentrations has been well documented (*see* Short 1987), the long-term indirect impacts of nutrient loading on the eelgrass community are not only negative but severe (Harlin and Thorne-Miller 1981; Dennison et al. 1993; Neckles et al. 1993).

The reason for the various responses by different algal forms to identical nutrient enrichment treatments in the mesocosms is not completely understood. However, the impact of animals in the process was apparent in 1989 and has been observed in field studies (van Montfrans et al. 1984; Williams and Ruckelshaus 1993) and in experimental studies (Howard and Short 1986; Neckles et al. 1993). The effect of animals in controlling the plant community was again evident during the shade-by-nutrient experiment in 1990. Judicious stocking of predatory fish (sticklebacks and pipefish) in the 1990 mesocosms controlled amphipods and resulted in a fairly balanced community of plant competitors in the enriched treatments under full light (Table 2). Fish and herbivorous amphipods as well as filter-feeding bivalves appeared to be important in regulating the dominant form of primary producer within the experimental treatments, representing top-down control of trophic levels (*see* Karr et al. 1992).

Overall, we found the primary effect of nutrient loading to be shading, as algal forms become more dominant at the expense of eelgrass. Shading resulted in a reduction in growth (per shoot and per m^2) and biomass, as did nutrient enrichment, but direct shading resulted in longer leaves, while enrichment resulted in shorter leaves. Our

results demonstrate the morphological and physiological adaptations of eelgrass bed structure in response to altered environmental conditions of light and nutrients.

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